Exhibit I

THG113: A Novel Selective FP Antagonist that Delays Preterm Labor

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$PGF_{2\alpha}$ is an important smooth muscle contractile agent that exerts significant effects on myometrium and is implicated in labor. THG113 was recently identified as a $PGF_{2\alpha}$ receptor (FP) antagonist. We characterized the specificity and selectivity of THG113, tested its effects on $PGF_{2\alpha}$-induced smooth muscle contraction, and assessed its efficacy in a model of endotoxin (LPS)-induced preterm labor. [^{125}I]THG113 bound specifically to FP-expressing but not to native (not expressing FP) HEK293 cells. In FP-expressing HEK293 cells, THG113 markedly reduced $PGF_{2\alpha}$-elicited phosphoinositide hydrolysis (IC_{50} 27 nM). Similarly, $PGF_{2\alpha}$-evoked microvascular (retinal) contraction was noncompetitively blocked (by >90%) by THG113. In contrast, contraction to agonists of homologous prostanoid receptors EP_1 and TP (17-phenyl-trinor PGE_2 and U46619) was unaffected (<1%) by high concentrations of THG113 (100 μmol/L); THG113 (100 μmol/L) also did not affect contraction to numerous other agents including platelet activating factor, endothelin, and angiotensin II. Force and duration of $PGF_{2\alpha}$-evoked contractions of myometrial strips of pig (non-pregnant, luteal phase) and mouse (immediately postpartum) were markedly reduced by THG113. In an endotoxin-induced preterm mouse model, lipopolysaccharide (50 μg intraperitioneal) injection at 16 days' gestation resulted in 100% delivery within 15 h; in contrast, 70% of those treated with THG113 (1 mg/day) delivered >24 h later (at 18 days' gestation; term: 19 days). In addition, in mice injected with lipopolysaccharide and treated 6 h later with THG113 (0.1 mg bolus followed by 1 mg/day) 40% delivered >48 h later. Fetuses of pregnant mice treated with THG113 were born alive, had higher birth weights (1.6 ± 0.1 *v* 1.4 ± 0.05 g), and appeared healthy. This study describes an effective and selective noncompetitive FP antagonist, THG113, which significantly delays preterm delivery; this provides the basis for future investigations for its use in tocolysis.

Actions of specific prostaglandin synthases results in the formation of 5 major prostanoids, PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2, and TXA_2. They exert their effects by acting on specific G protein-coupled receptors; PGE_2 via $EP_{1\text{-}4}$, $PGF_{2\alpha}$ via FP, PGD_2 via DP, PGI_2 via IP and TXA_2 via TP.[1] Diverse effects of PGs in mammalian physiology are well described, and different prostaglandins produce opposing effects on smooth muscle contraction; for instance, PGI_2 and PGD_2 are relaxants, whereas $PGF_{2\alpha}$ and TXA_2 are constrictors; PGE_2 has different effects depending on the receptor distribution and composition in the tissue. In many tissues and conditions, more than 1 type of prostaglandin is formed. At present, inhibiting cyclooxygenase activity by nonsteroidal anti-inflammatory drugs (NSAIDS) is the only available means of therapy in conditions where blockade of prostanoid action is desired. But, nonselective inhibition of prostanoid action may produce undesired actions. Thus prostanoid receptor-selective drugs offer a more effective therapy in variety of medical conditions.

$PGF_{2\alpha}$ is the most effective smooth muscle contractile prostaglandin.[1] $PGF_{2\alpha}$ stimulates myometrial contraction in several species[2,3] as

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other prostaglandins, namely PGE_2, may cause relaxation in lower segments of the uterus.[2,4] Indeed, $PGF_{2\alpha}$ plays a pivotal role in parturition as indicated by several lines of evidence: 1) A significant rise in $PGF_{2\alpha}$ levels in amniotic fluid during early labor[5,6]; 2) $PGF_{2\alpha}$ stimulates uterine contractility[2] and induces labor[7]; 3) $PGF_{2\alpha}$ receptor expression augments markedly during term and especially preterm parturition as part of the contraction-associated proteins (which include actin, myosin, connexin-43, receptors for oxytocin and prostaglandins, and possibly COX-2)[4,8-11]; 4) Effects of the major uterotonic oxytocin are partly mediated by $PGF_{2\alpha}$ and the latter in turn augments oxytocin receptor expression[12,13] such that oxytocin and prostaglandins are closely intertwined[3]; 5) Inhibition of prostaglandin formation reduces myometrial contractions and prolongs labor[14-16]; and 6) Disruption of the $PGF_{2\alpha}$ receptor gene leads to failure of animals to go into labor.[17]

Although numerous $PGF_{2\alpha}$ receptor agonists have been developed,[18,19] until now there have been no selective antagonists. THG113 is a rationally designed compound and was recently reported to exhibit selective FP receptor antagonism.[20] Therefore, we planned to characterize the specificity and selectivity of THG113 on FP-expressing cells and on smooth muscle and determine its efficacy in a model of endotoxin-induced preterm labor.

Materials and Methods

Transfection of FP Receptor cDNA in Human Embryonic Kidney 293 Cells

The full-length coding sequence of human FP was subcloned into the mammalian expression vector pRcCMV (Invitrogen) using methods reported previously.[21] The plasmid was prepared with a Qiagen plasmid preparation kit and 2 μg of DNA were transfected into human embryonic kidney (HEK) cells with Lipofectamine (Life Technologies, Burlington, Ontario, Canada). The transfectants were selected by using G418 (1 mg/mL) for 2 weeks. Polyclonal population of FP transfectant cell line (FP/293) were grown in Dulbecco's Modification of Eagles Medium complete medium containing G418 (0.2 mg/mL) at 37°C in a humidified atmosphere at 5% CO_2 in air.

THG113 Binding Assay

FP/293 cells were trypsinized and washed with binding buffer (sodium phosphate buffer 0.01 mol/L pH 7,4; NaCL 0.1 mol/L; $MgCl_2$ 0.002 mol/L; acetylsalicylic acid 0,02% p/v; benzamidine 0.001 mol/L; EGTA 0.0005 mol/L; glucose 0.9 mg/mL and protease inhibitor cocktail (Roche Biochemical, Burlington, NC). Binding was performed at 37°C for 30 min with 10^5 cells, [^{3}H]$PGF_{2\alpha}$ (20 nmol/L final; specific activity 212 Ci/mmol) or with [^{125}I]THG113 (100,000 cpm final) and 10^{-5} to 10^{-10} mol/L of cold $PGF_{2\alpha}$ or THG113. The reaction was started by adding the cells for a final reaction volume of 100 μL and stopped by 3 washes of 4 mL of stop buffer (cold Tris-HCl 50 mmol/L pH 7.4). The reaction mix was then filtered through GF/B filters. Radioactivity on filters was counted with a scintillation counter. Specific binding was the difference between the binding in the absence and in the presence of unlabeled ligands.

Phosphoinositide Hydrolysis Assay

FP/293 grown in 12 well plates were labeled with 1-2 uCi/mL [^{3}H]-myo inositol (17 Ci/mmol; Amersham Canada) overnight. The cells were preincubated in DMEM containing 10 mmol/L LiCl with different concentrations of THG113 for 30 minutes at 37°C. Cells were then stimulated with 100 nmol/L $PGF_{2\alpha}$ for 30 min at 37°C and the reaction was terminated by addition of 0.5 volume of NaOH (0.1 N), followed by acidification with 0.2 N formic acid. Cells were collected by scraping and 0.4 mL each of methanol and chloroform were added. Total inositol phosphates were separated by using Dowex AG1X8 (formate form) and 1.2 mol/L ammonium formate in 0.1 N formic acid as the eluant. Radioactivity of phosphoinositides was determined by liquid scintillation method.

Vascular Contraction Preparations

Retinal eyecup preparations for measuring vasomotor responses were prepared as described.[22,23] FP receptor densities were augmented by treating newborn pigs with ibuprofen (30 mg/kg every 8 h for 24 h).[24] Eyes were immediately removed and placed in ice-cold Krebs buffer of the following composition (mmol/L): NaCL, 120; KCL, 4.5; $CaCL_2$, 2.5; $MgSO_4$, 1.0; $NaHCO_3$, 27; KH_2PO_4, 1.0; sodium

edetate, 0.01; glucose, 10; and heparin (1.5 U/mL). After removal of the anterior segment the eyecup was pinned to a wax support in a 20-mL Krebs buffer bath equilibrated with 95% O_2 and 5% CO_2, and maintained at 37°C and pH 7.35-7.45. Dose-responses of various vasoactive compounds in the presence/absence of THG113 on retinal vasomotricity were recorded by video imaging as described in detail.[22-24] The outer vessel diameter was recorded with a video camera mounted on a dissecting microscope (Zeiss M 400, Thornwood, NY) and the responses were quantified by a digital image analyzer (Sigma Scan Software; Jandel Scientific, Corte Madera, CA). Vascular diameter was recorded before and 5 min after the topical application of the agonist.

Myometrial Contraction Preparations

Porcine uterine tissues were collected from animals during luteal phase (by ovarian examination) from a local abattoir (St-Hélène, Québec). Uterus from mice were obtained from animals immediately after term delivery. Myometrial strips (2 to 3-mm wide and 1 to 2-cm long) from both species were suspended in organ baths containing Krebs buffer equilibrated with 21% oxygen at 37°C as described previously.[25-27] Initial tension was set at 2 g. After 1 h of equilibration, changes in mean basal tension, as well as peak, duration and frequency of spontaneous contractions in response to added agents were recorded with a Kent digital polygraph system.

Endotoxin-induced Preterm Labor Mouse Model

Timed-pregnant CD-1 mice at 16 days' gestation (term: 19.2 days) were anesthetized with ketamine (100 mg/kg intramuscular) and xylazine (5 mg/kg intramuscular). Primed osmotic pumps (Alzet pump, Alzet, Cupertino, CA) containing either saline or THG113 (1 mg/day/animal) were subcutaneously implanted on the backs of the animals; infusion of the peptide was immediately preceded by bolus injection of THG113 (0.1 mg/animal intraperitioneal). Within 15 min after placement of the pumps, the animals were injected with lipopolysaccharide ([LPS] E-coli endotoxin, 50 μg/animal intraperitioneal) to mimic the infectious component in human preterm labor.[28,29] In a separate group of animals LPS was injected 4 to 7 h prior to administration of THG113. Animals were in-

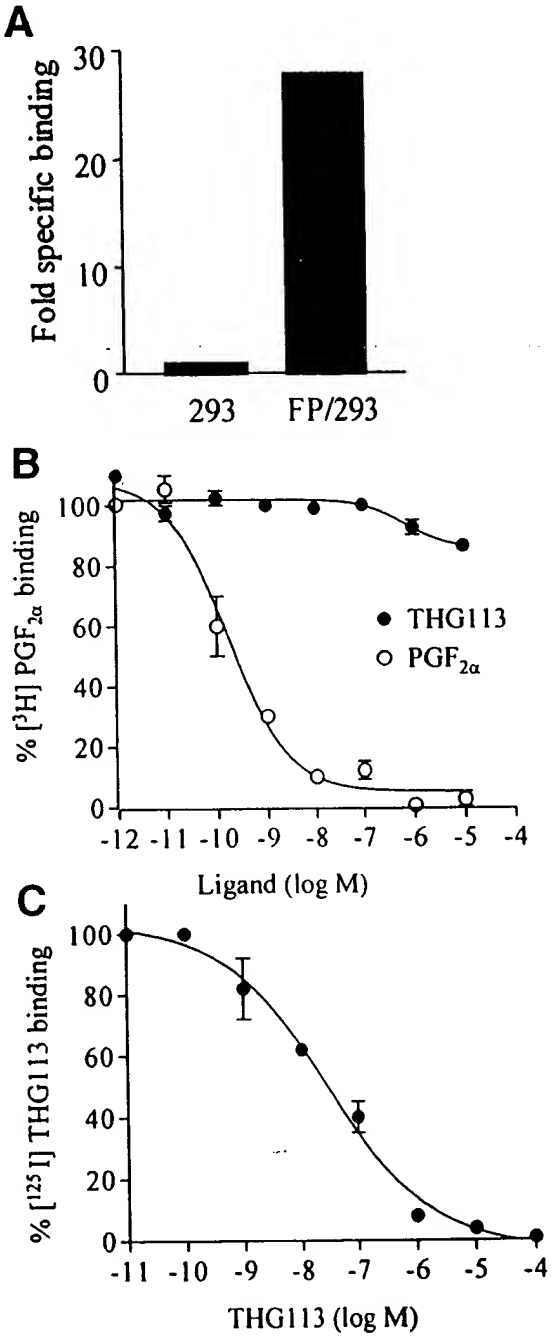

Figure 1. Binding of THG113 to FP/cells. A) Specific binding of [^{125}I]THG113 to FP/293 cells. B) Displacement of [^{3}H]$PGF_{2\alpha}$ by $PGF_{2\alpha}$ and THG113. Values are mean ± SEM. C) Displacement of [^{125}I]THG113 by THG113.

spected every hour for the first 18 h and every 2 h thereafter to document the timing of birth.

Statistical Analysis

Data were analyzed by one- or two-way ANOVA factoring for treatment group or drug concentration, followed by the Tukey-Kramer method for comparison among means. Nonparametric analysis was performed by Fisher exact test. Statistical significance was set at $P < .05$.

Results and Discussion

Binding of THG113

FP/293 cells exhibited specific binding of [^{125}I]THG113; [^{125}I]THG113 did not bind to parent HEK293 cells (Fig 1C). Binding of [^{125}I]THG113 was fully displaced by THG113 with an IC_{50} = 35 nM (Fig 1B). To determine if THG113 bound to the same site as $PGF_{2\alpha}$ on FP/293 cells, displacement of specifically bound [^{3}H]$PGF_{2\alpha}$ by THG113 was measured in FP/293 cells. [^{3}H]$PGF_{2\alpha}$ was negligibly displaced by THG113 but fully displaced by $PGF_{2\alpha}$ (IC_{50} = 0.16 nmol/L; Fig 1B), suggesting distinct binding sites for THG113 and $PGF_{2\alpha}$ on FP.

Effect of THG113 on Inositol Phosphate Production

FP/293 expressed a functional FP receptor; accordingly, $PGF_{2\alpha}$ augmented phosphoinositide hydrolysis (Fig 2A).[1,30] THG113 inhibited $PGF_{2\alpha}$-induced inositol phosphate formation with an IC_{50} = 27 nmol/L (Fig 2B); THG113 (100 nmol/L) maximally inhibited inositol phosphate generation (Fig 2C).

Effects of THG113 on Vasomotor Response to $PGF_{2\alpha}$

The efficacy of THG113 was tested in newborn porcine retinal vasculature known to express FP after ibuprofen treatment.[24] $PGF_{2\alpha}$ elicited a strong vascular contraction; this effect was nearly

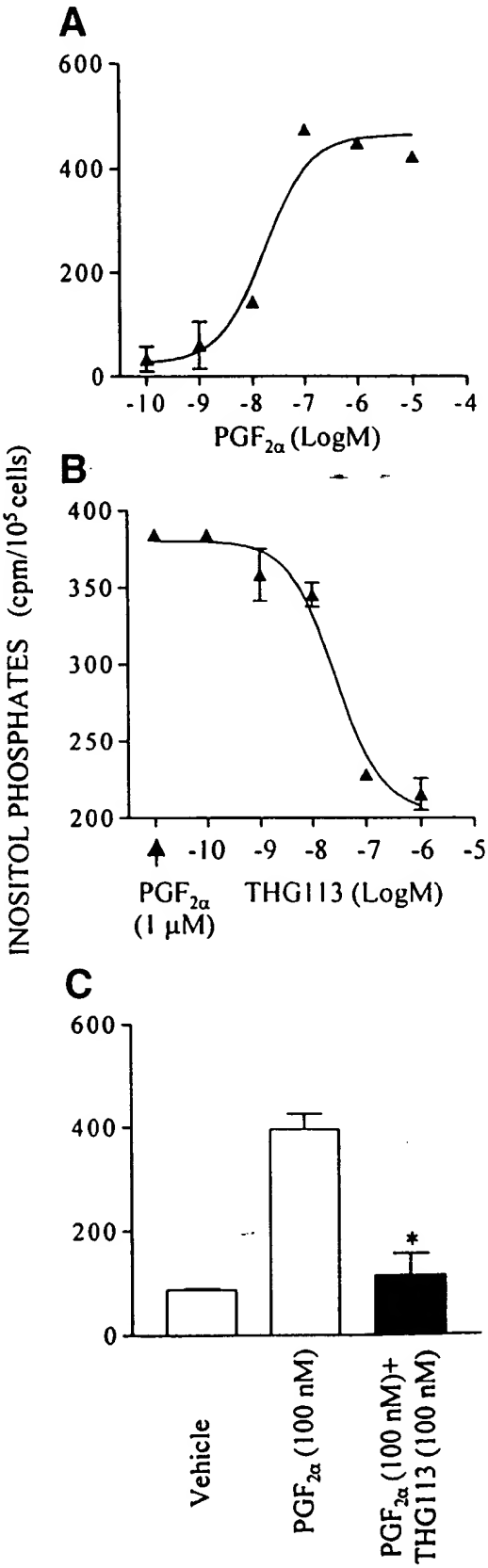

Figure 2. Effect of THG113 on $PGF_{2\alpha}$-induced inositol phosphate production in FP/293 cells. A) Effect of $PGF_{2\alpha}$ on inositol phosphate production. B) and C) Inhibition of $PGF_{2\alpha}$-induced inositol phosphate production by THG113. Values are mean ± SEM. *$P < .05$ compared to corresponding cells untreated with THG113.

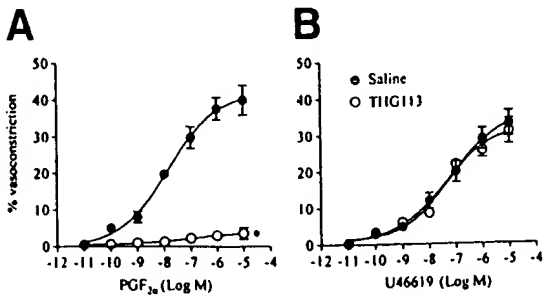

Figure 3. Effect of THG113 on A) $PGF_{2\alpha}$- and B) U46619-induced retinal vasoconstriction.

completely blocked by 100 μM THG113 (Fig 3A) even at high concentrations of $PGF_{2\alpha}$; the vasomotor responses to $PGF_{2\alpha}$ were fully recovered by the removal of THG113 from the buffer (data not shown). Distinct site of binding (Fig 1B) and the inability of the agonist to displace THG113 (Fig 3A) suggested a noncompetitive mode of inhibition of FP receptor by THG113. In contrast, contraction to U46619, the TXA_2 mimetic and an agonist of TP receptor (homologous to FP),[30] was unaffected by THG113 (Fig 3B and Table 1).

We further evaluated the selectivity of THG113 to FP by measuring the retional vasomotor responses of various agents (Table 1). High concentrations of THG113 (100 μmol/L) did not antagonize effects of numerous constrictors including other lipids, catecholamines, peptides as well as to sarcoplasmic calcium ATPase inhibitor BHQ (Table 1); more importantly, THG113 minimally inhibited constriction to 100 nmol/L 17-phenyl trinor PGE_2, the agonist of EP1-most homologous prostanoid receptor to FP.[30] These data suggested that THG113 is a selective inhibitor of FP receptor.

Table 1. Selective Inhibition of Retinal Vasoconstriction by THG113

Agonist (Target)	*Percent Inhibition of Retinal Vasomotor Responses*
$PGF_{2\alpha}$ (FP)	90.4
17-phenyl trinor PGE_2 (EP_1)	5.0
U46619 (TP)	<1
C-PAF (PAF-R)	4.0
Phenylephrine (α1 adrenoceptor)	<1
Urotensin II (GPR14)	<1
Endothelin (ET)	<1
Angiotensin II (AT)	<1
BHQ (sarcoplasmic Ca^{++} ATPase)	<1

NOTE.
Constriction of FP-containing retinal vasculature[22,24] was studied by video-imaging technique, as described in Materials and Methods. Dose-response to agonists (0.1 umol/L) were studied in absence and presence of THG113 (100 umol/L).

Inhibition of Myometrial Contractility by THG113

In nonpregnant porcine myometrial tissues, THG113 diminished $PGF_{2\alpha}$ (1 μmol/L)-in-

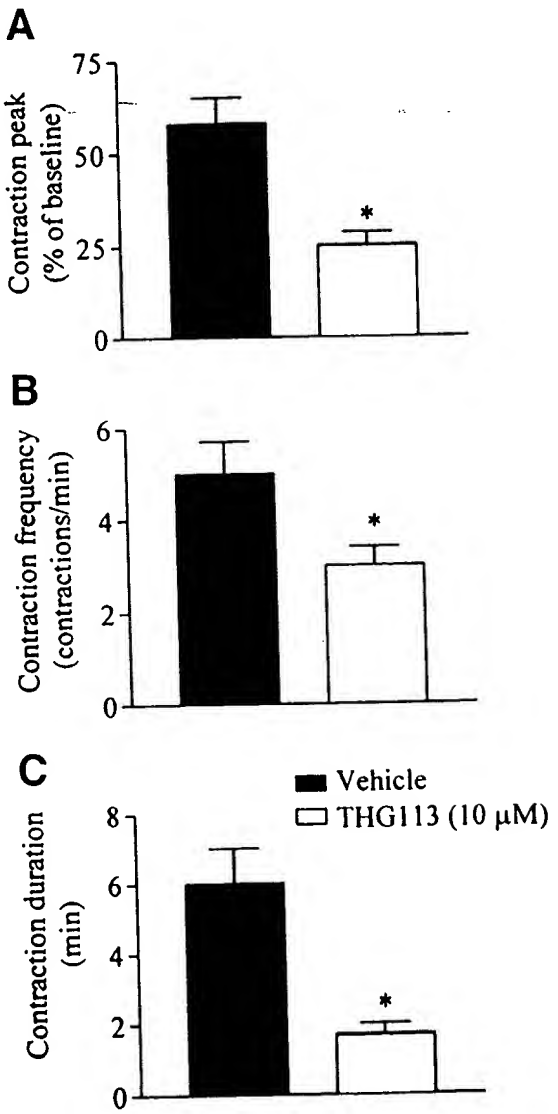

Figure 4. Modulation of $PGF_{2\alpha}$ (1 μmol/L)-induced porcine myometrial contractions by THG113. Values are mean ± SEM. *$P < .05$ compared to vehicle-treated tissues.

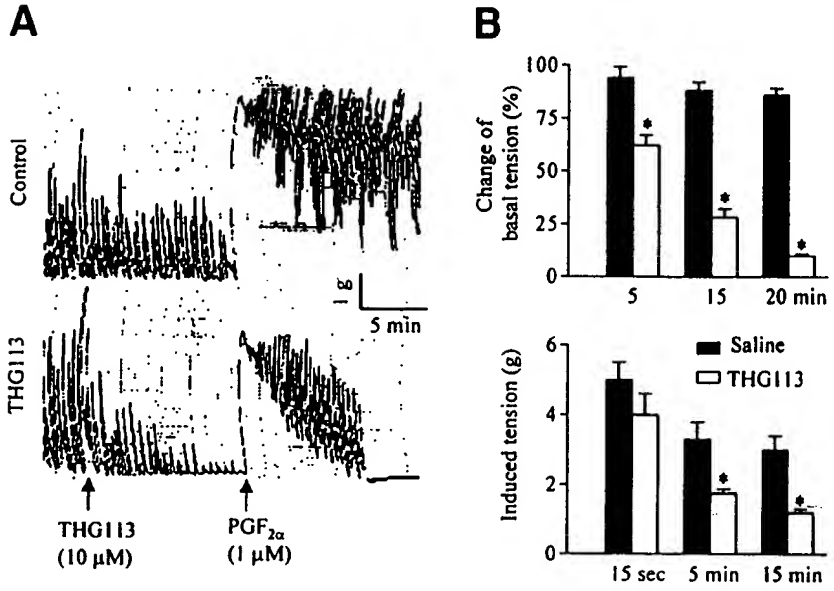

Figure 5. Reduced contraction of immediate postpartum mouse myometrium by THG113. A) Tracings of spontaneous and $PGF_{2\alpha}$ (1 μmol/L)-induced myometrial contractility of tissues treated with THG113 or saline (control). B) Histogram depicting time-dependent change in pre- (basal) and post-$PGF_{2\alpha}$ (1 μM)-evoked myometrial tension in saline- and THG113-treated tissues. Values are mean ± SEM. $^*P < .05$ compared to corresponding value in saline-treated tissues.

duced peak, duration and frequency of myometrial contractions (Fig 4). To test the effect of THG113 on uterine contractility in the process of labor, $PGF_{2\alpha}$ responses on myometrial strips from CD-1 mice immediately after delivery were measured in organ baths. THG113 blocked spontaneous myometrial contractions, but only slightly reduced $PGF_{2\alpha}$-induced tension; however, THG113 dissipated $PGF_{2\alpha}$-induced contractions of mouse myometrium more rapidly than those observed after saline treatment (Fig 5).

Effect of THG113 on LPS-induced Premature Delivery

All saline-treated CD-1 mice delivered within 15 h after LPS injection on day 16 of gestation (term: 19 days) (Figs 6A and B). Treatment with THG113 (1 mg/day/animal) significantly delayed delivery such that 30 h later one third of animals still had not delivered, and 48 h later (corresponding to 19 days' gestation) all animals had delivered; the dose used exerted maximum efficacy as higher doses did not further delay delivery. Mouse pups from mothers treated with THG113 had increased birth weights (Fig 6C), and appeared healthy. Comparably, in mice treated with THG113 6 h after LPS injection, THG113 delayed delivery by 48 h (18.5 days gestation) in 40% of the mice (Fig 7).

A significant role for progesterone has been suggested in the onset of labor in rodents.[17] $PGF_{2\alpha}$ is a luteolytic agent; this results in decreased progesterone, which in turn induces parturition in rodents.[31] Ovariectomy of the FP-deficient mouse at 19 days' gestation induces delivery 24 h later.[17] On the other hand, in humans, concentrations of progesterone that arise from placenta[31] decrease only after placental delivery,[32] and $PGF_{2\alpha}$ is a potent uterotonic agent.[7] To distinguish the leuteolytic and uterotonic actions of FP receptor, the effect of THG113 on mice treated with the progesterone receptor inhibitor RU486 was determined.[33] RU486 (0.1 mg/animal ip) administered on day 17 of gestation caused delivery within 12 h; on the other hand, in mice treated concomitantly with THG113 delivery was delayed until 24 h after RU486 administration (data not shown). These findings support a role for myometrial FP in parturition in rodents, consistent with other reports.[3,7]

A number of other interactive factors in particular, cytokines, oxytocin, and other prostanoids may contribute to the process of parturition.[31,34] Indeed, progesterone plays a key role in down-regulating FP expression,[35] which in turn governs the expression of oxytocin receptor[17] as well as that of COX-2 in myometrium; expression of COX-2, which is FP receptor-dependent,[17] results in the synthesis of $PGF_{2\alpha}$, as well as of PGE_2, which exerts important uterotonic activities[36] largely via EP_3 receptors.[2,37] Oxytocin seems to participate mainly in term labor, whereas $PGF_{2\alpha}$ exerts a predominant role

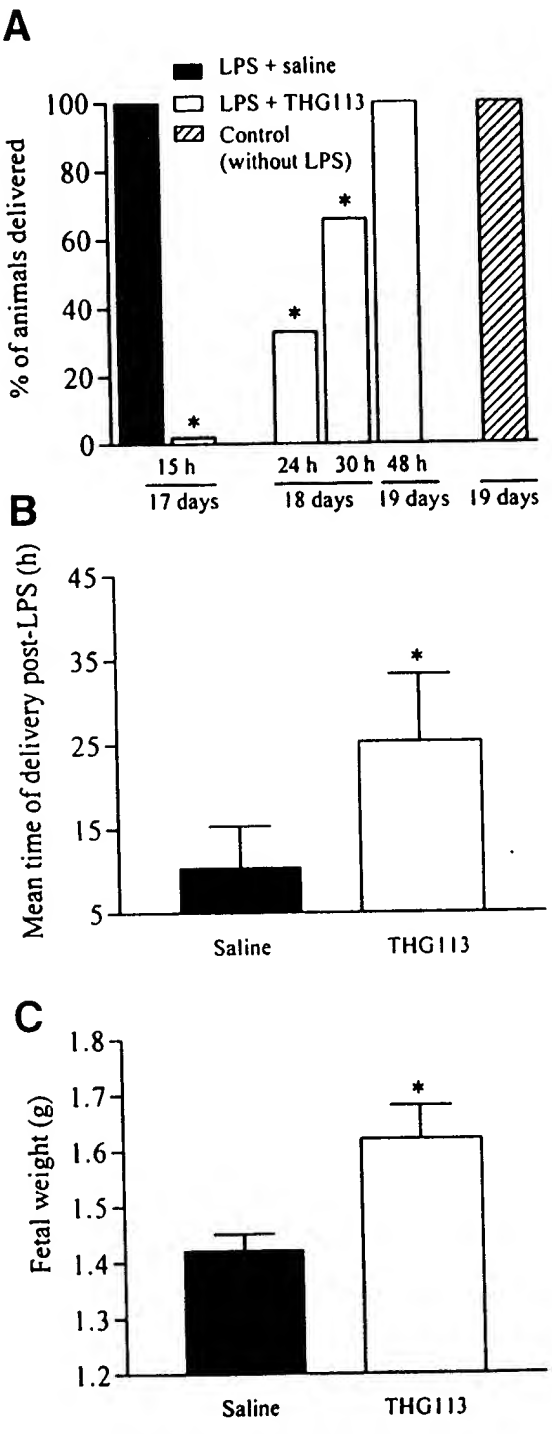

Figure 6. Tocolytic effect of THG113 in LPS-induced preterm labor in mice. Pregnant mice were injected at 16 days' gestation (term: 19 days) with LPS with or without concomitant treatment with THG113, as described in Materials and Methods. A) Percent of animals delivered; hatched bar refers to control animals not treated with LPS. Hours refer to time after LPS treatment and days to gestational age. B) Average delivery time after LPS; values are mean ± SEM. C) Weight of pups at delivery; values are mean ± SEM. $*P < .05$ compared to saline-treated mice.

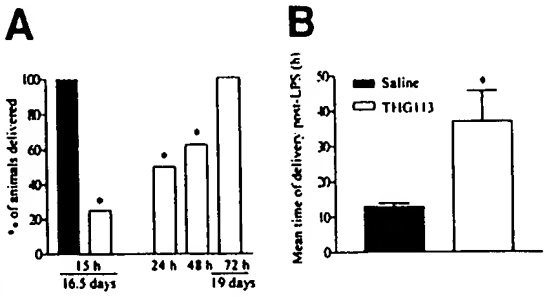

Figure 7. Tocolytic effect of THG113 4 to 7 h after administration of LPS to induce preterm labor. A) Percent of animals delivered. Hours refer to time after LPS treatment and days to gestational age. B) Average delivery time after LPS; values are mean ± SEM. $*P < .05$ compared to saline-treated mice.

in preterm labor.[8] The tocolytic efficacy of THG113 is consistent with the latter inference disclosing a critical role for FP in preterm parturition, particularly in preterm (present data and ref 8).

In conclusion, we have characterized THG113 as a selective, noncompetitive, reversible, and potent FP receptor antagonist. THG113 inhibited spontaneous as well as $PGF_{2\alpha}$-induced myometrial contractions, and prolonged gestation in an endotoxin-induced preterm labor mouse model, which mimics the infectious component of preterm labor.[28,29] Given the limited distribution of FP receptors in the organism,[18] the major role of FP in parturition,[17] and the relative efficacy of inhibitors of COX,[14,16,38] including of COX-2[36] in tocolysis, albeit at risk of complications,[39,40] targeting selectively FP receptor may produce efficacious and safe tocolytics for preterm labor; in this regard, THG113 is an interesting prototype.

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SEMINARS IN PERINATOLOGY

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INTRODUCTION

THE INTERNATIONAL Perinatal Collegium is a body of colleagues from many parts of the world that meets every 2 years to exchange new research findings in the areas of perinatology and neonatology. The organizers of this group are Dr Bill Oh from Providence, RI, Dr David Schiff from Edmonton, Alberta (Canada), and Dr Paul Vert from Nancy (France). The meeting is made possible by the generous support of Mead Johnson Nutritionals. The most recent meeting of this Collegium was held on Marco Island, FL, from July 21-25, 2001, when 50 members and guests from 15 countries came together for a lively and provocative scientific exchange. Eleven papers presented at the meeting were selected for inclusion in this issue of *Seminars in Perinatology*.

The first 2 papers in this issue focus on eicosanoid biology. The cyclooxygenase (COX) inhibitor indomethacin is commonly used as a tocolytic, but its efficacy is limited and its use is associated with significant adverse effects in the fetus and newborn. Dr K.G. Peri and colleagues have taken a different approach to the prevention of premature labor with their investigations of a receptor antagonist for one of the COX products, $PGF_{2\alpha}$, which is known to play a pivotal role in parturition. In their article, they describe the pharmacological properties of this receptor antagonist, THG113, and show its ability to delay preterm delivery in a mouse model of endoxin-induced premature labor.

Indomethacin is also used to prevent or treat symptomatic patent ductus arteriosus in newborn premature infants. The use of indomethacin in this context is associated with significant adverse effects, particularly decreased renal function. Another COX inhibitor, ibuprofen, has been recommended as an alternative to indomethacin because several clinical trials have shown less effect on renal function with ibuprofen than with indomethacin. However, Dr Jean-Pierre Guignard showed in 6-day-old rabbits that ibuprofen had adverse effects on renal function that were no different from those occurring with indomethacin and aspirin. These results emphasize that neonatologists should be concerned about adverse effects on renal function regardless whether indomethacin or ibuprofen is chosen for use in the management of symptomatic patent ductus arteriosus.

The question whether we are doing more harm than good when we administer 100% oxygen to asphyxiated infants in the delivery room has received further examination by Dr Máximo Vento and colleagues. They report evidence of increased oxidative stress in asphyxiated infants resuscitated with 100% oxygen. These findings raise the possibility that hypoxic ischemic encephalopathy, which involves reperfusion-generated free radical injury, might be further aggravated by resuscitation with high concentrations of inspired oxygen. Neonatologists may be exposing infants inadvertently to the adverse effects of oxygen by means of another common neonatal intensive care unit (NICU) intervention—blood transfusion. In their article, Dr Virginie De Halleux and colleagues show that the decreased oxygen affinity after transfusion with adult red blood cells increases the amount of oxygen available to the immature tissues of preterm infants. They go on to offer the provocative recommendation that lowering oxygen saturation targets after a transfusion may protect against hyperoxygenation and free radical damage.

Another toxin to the immature neonate is

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bilirubin. By using cultured neurons from the developing rat brain, Dr Stéphanie Grojean and colleagues show the adverse interaction of free bilirubin and hypoxia on cell viability and apoptosis, protein synthesis, and energy metabolism. Not only did the combination of bilirubin with hypoxia result in stronger detrimental effects on neurons than bilirubin or hypoxia alone, bilirubin was also shown to prevent the adaptive cell response to sublethal hypoxia. In view of these findings, would Dr De Halleux group's recommendation for lower oxygen saturation targets also apply after an exchange transfusion for hyperbilirubinemia?

Mechanical ventilators now in use in NICUs often display a variety of electronically derived information such as real-time flow-volume and pressure-volume graphs. Dr Hugo Devlieger and colleagues have added a third graph—flow *v* pressure—to the ventilatory information available at the bedside of infants being mechanically ventilated. In their article, they describe how the flow-pressure graph can be used to detect subtle changes in infant-ventilator interaction. This information could lead to further refinements in the bedside management of mechanical ventilation and help reduce some of the lung injury inflicted by mechanical ventilation.

Most neonatologists think of nitric oxide as a molecule involved in the modulation of pulmonary vascular tone. In their article, Dr Richard J. Martin and colleagues call our attention to the effects of NO on airway smooth muscle tone. Of particular interest is that the ability of endogenously released NO to oppose airway constriction may be impaired in response to hyperoxia. This and other projected benefits of inhaled NO on airway function may become recognized as a result of clinical trials currently underway evaluating the efficacy of inhaled NO in the prevention and treatment of BPD.

Achieving adequate nutrition in premature infants, especially those with compromised gastrointestinal function, remains a significant challenge for neonatologists. Often, partially hydrolyzed formulas are used to facilitate intestinal absorption of nutrients. However, these formulas result in decreased nitrogen and calcium absorption rates compared with standard premature formulas. To address this problem, Dr Jean-Charles Picaud and colleagues evaluated a new formula with modified nitrogen and calcium sources. In their article, they report that an appropriate nitrogen retention and plasma amino acid profile can be achieved with the new formula.

It has been suspected for decades that the risk of respiratory distress syndrome (RDS) may be affected by genetic factors. Two papers in this issue address this question. In their article, Dr Loekie van Sonderen and colleagues present data from a twin study supporting this suspicion. They showed that RDS occurred more frequently in both twins when the twins were monozygotic than when the twins were dizygotic. In the other article, Dr Mikko Hallman and colleagues were unable to show any major direct genetic influence when they screened nearly one million birth records of Finnish origin to identify premature multiple births with at least one RDS infant. Their findings appear to contradict the van Sonderen report and the growing body of evidence that certain genetic polymorphisms in surfactant proteins A and B increase RDS risk. However, they go on to argue that this apparent conflict can be resolved by taking into consideration birth order of twins and differences in environmental factors such as cytokine exposure between the first and second born twin.

In the final article, Dr Bobbi J. Byrne and colleagues present epidemiological evidence of a decline in the risk of bronchopulmonary dysplasia in their NICU since the beginning of 1991. Prior to 1991, improving survival of very low birth weight infants was accompanied by a proportional increase in the incidence of BPD. The reversal of this trend in the past decade was associated with a variety of changes in clinical practice. If these findings are representative of other NICUs, they signify an important reduction in the impact of BPD as one of the costly sequelae of prematurity.

We hope that this selection of papers from the 2001 International Perinatal Collegium will be of interest to the readership of *Seminars in Perinatology*.

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Guest Editor